Articles

Clinical Sequelae of Hepatitis C Acquired From Injection Drug Use

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We determined the course of hepatitis C infection in 125 patients with a history of injection drug use. The mean age at presentation was 43.5 years, and the mean age of initiating injection drug use was 23.1 years. Fatigue and hepatomegaly were present in as many as 60% of patients. All had antibodies to the hepatitis C recombinant protein C25, and 99% were positive for hepatitis C virus RNA. After the initial workup, 33 (26%) patients had chronic hepatitis, 46 (37%) had chronic active hepatitis, 45 (36%) had cirrhosis, and 1 (0.8%) presented with hepatocellular carcinoma. During follow-up, hepatocellular carcinoma developed in 2 other patients. In 74 patients with a 1-year history of injection drug use, the mean number of years to the development of chronic hepatitis, chronic active hepatitis, cirrhosis, and hepatocellular carcinoma were 15.6, 17.6, 19.4, and 26.3 years, respectively. In this subgroup of patients, heavy alcohol abuse did not appear to influence the progression of liver disease. The 2-year case-fatality rate was 2%. Our findings indicate that hepatitis C is a progressive disease, but only a few died during the average 20.4 years after the initiation of injection drug use. Antiviral treatment to eradicate the virus and halt the progression of disease is indicated in this group of patients.

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Persons who indulge in injection drug use are prone to infection by blood-borne agents such as the human immunodeficiency virus (HIV), the hepatitis B virus (HBV), and the hepatitis C virus (HCV). In cases of HCV, seroprevalence rates of antibody to HCV in injection drug users seeking medical treatment in an emergency department in Baltimore, Maryland, were 83%, and in those involved in drug treatment programs in Sacramento, California, they were 72%.[1,2] These rates of positivity for antibody to HCV (anti-HCV) in two geographic areas of the United States are similar to those reported in injection drug users in Spain (70%), United Kingdom (81%), Australia (86%), and Amsterdam (74%).[3-6] Comparative studies on the three blood-borne viruses showed that anti-HCV was detected more frequently than hepatitis B surface antigen (6% to 8%) or antibody to HIV (1% to 27%) in injection drug users.[1,2] Therefore, chronic HCV infection is an important health problem in this group of patients.

A report from the Centers for Disease Control and Prevention showed that injection drug use accounted for 36% of the cases of acute hepatitis C in the United States.[7] After acute HCV infection, persistently elevated serum alanine aminotransferase (ALT) levels were detected in as many as 67% of patients. In some patients in whom serum ALT levels returned to normal after acute infection, HCV RNA was still detectable, indicating continual viral replication even in the absence of biochemical evidence of liver inflammation.

There have been few follow-up studies in patients with chronic hepatitis C infection. Two studies showed that the histopathology was less severe and the clinical progression may be slower in patients who acquired their chronic hepatitis C from injection drug use than through blood transfusion.[8,9] Two other reports on patients with posttransfusion hepatitis C indicate that the progression of chronic liver disease was slow and that few had complications after 16 to 18 years of follow-up.[10,11] In contrast, our recent report on a cohort of patients referred to a tertiary care center showed that posttransfusion chronic hepatitis C was a progressive disease and may result in death from either liver failure or hepatocellular carcinoma.[12] To further elucidate the natural history of chronic hepatitis C, we report herein the clinical, biochemical, and histologic findings of patients who acquired their HCV infection through injection drug use.

Patients and Methods

Between December 1981 and October 1994, 170 patients with chronic hepatitis who gave a history of injection drug abuse were evaluated at the Liver Center, Huntington Memorial Hospital, Pasadena, California. All tested positive for antibodies to HCV by methods

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The hepatitis C antibody assays for this study were performed by George Kuo, PhD, and David Chien, PhD, from Chiron Corporation, Emeryville, California.

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ABBREVIATIONS USED IN TEXT

AFP = α-fetoprotein
ALT = alanine aminotransferase
anti-HBc = hepatitis B core antibody
anti-HBs = hepatitis B surface antibody
anti-HCV = antibody to HCV
HBsAg = hepatitis B surface antigen
HBV = hepatitis B virus
HCV = hepatitis C virus
HIV = human immunodeficiency virus

described later. Of these patients, 45 were excluded from study because of a coexisting history of blood transfusion and either coinfection with HBV or with HIV. Also, patients with other chronic liver diseases such as hemochromatosis and autoimmune chronic hepatitis were excluded. Thus, 125 patients with injection drug use–associated chronic hepatitis C were analyzed for the following study. Of these, 115 (92%) were referred by their private physician for the following reasons: 108 had abnormal liver test values or a positive anti-HCV antibody test, 6 had symptoms and signs of chronic liver disease, and 1 was referred for evaluation of a liver mass. The remaining 10 patients (8%) were self-referred either from a drug rehabilitation program or because of an abnormal ALT level or a positive anti-HCV antibody test after donating blood.

Liver biopsies were obtained from 112 of the 125 anti-HCV–positive patients. In the remaining 13 patients, liver biopsies were not done because of abnormal coagulation tests; all 13 patients had signs of cirrhosis. Liver biopsies were interpreted according to established histologic criteria, and four liver disease categories were described.[13] Chronic hepatitis had features of portal lymphoid hyperplasia, preservation of the limiting plate, and focal hepatocytolysis. This lesion was formerly referred to as chronic persistent hepatitis.[14] Chronic active hepatitis was diagnosed by the presence of piecemeal necrosis and parenchymal inflammation with or without bridging necrosis. Cirrhosis was characterized by the addition of nodular formation to the above changes. The diagnosis of hepatocellular carcinoma was made by histologic features or by findings of elevated α-fetoprotein (AFP) levels and radiographic changes consistent with this cancer.[15] During follow-up, all patients were screened for hepatocellular carcinoma by testing for AFP levels every six months and with a yearly ultrasound examination of the liver.

A subgroup of patients who gave a history of injecting drugs for a period of a year or less were questioned about alcohol intake. Each was asked to fill out a questionnaire that estimated the time period, type, and the amount of alcohol consumed. The lifetime consumption of alcohol in kilograms was estimated by using the following criteria: 12 oz beer = 4 oz wine = 1 oz liquor = 13 grams of alcohol.[16]

Laboratory Tests

The sera of patients were tested for HCV RNA by polymerase chain reaction as previously described.[14] All patients were tested for HCV antibodies by Chiron Corporation assays.[17] The antigens used were the chimeric C25 protein, which included C22, C100-3, and C33C, and the individual HCV recombinant proteins C22, E1, E2, NS3, C100-3, and NS5. The liver tests were measured by the Hitachi 747, Boeringer Manuheim, Indianapolis, Indiana. The AFP was assayed by Nichols Institute, San Juan Capistrano, California. The upper limit of normal for AFP was 18 μg per liter.

Hepatitis B Antibody Tests

Antibodies to hepatitis B surface antigen (anti-HBs) and to hepatitis B core antigen (anti-HBc) were measured by radioimmunoassay (AusAb and CorAB, Abbott Laboratories, North Chicago, Illinois).

Analysis

Data for each study variable were analyzed for the arithmetic mean and standard deviation of the mean. Statistical analysis was performed by the *t* test; all *P* values are two-tailed.

Results

Clinical Characteristics of Patients

During the first visit to the Liver Center, the average age of the 125 patients with a history of injection drug use was 43.5 years (range, 28 to 70 years). The average age at the time of initiating self-injection of drugs was 23.1 years (range, 8 to 60 years). There were 37 women and 88 men. The ethnic background of the 125 patients included 102 white, 3 Asian, 15 Hispanic, and 5 African American. The mean follow-up after presentation was 21 months (range, 1 to 115 months).

The estimated length of time of injection drug use in the 125 anti-HCV–positive patients varied. A total of 74 patients (59%) admitted to self-injecting drugs for a period of only one year or less, 23 patients (18%) for two to five years, and 13 patients (10%) for six to ten years. In addition, ten patients self-injected drugs for 20 years, four patients for 30 years, and one patient for 40 years. An episode of acute hepatitis characterized by jaundice, fatigue, and dark urine within six months of injection drug use was reported by 35 patients (28%).

Hepatitis C Viral Antibodies and Hepatitis C Viral RNA

Analysis of the antibody profiles to recombinant HCV antigens showed that all 125 patients had antibodies to C25 chimeric protein. Antibodies to C22, NS3, NS5, and C100-3 were detected in 98%, 95%, 76%, and 74% of patients, respectively. Antibodies to structural proteins E1 and E2 were found in 56% and 45% of patients. The HCV RNA was positive in 124 of 125 (99%) of these patients.

Hepatitis B Viral Antibodies

Evidence for past hepatitis B infection was present in 42 of 92 (46%) anti-HCV–positive patients who had HBV antibody tests performed. Anti-HBs only was

TABLE 1.—*Presenting Symptoms and Signs by Liver Disease Category of 125 Patients With Hepatitis C Acquired by Injection Drug Use*

Symptom or Sign	Chronic Hepatitis (n = 33)* No. (%)	Chronic Active Hepatitis (n = 46)* No. (%)	Cirrhosis*† (n = 46) No. (%)	Total No. of Patients (n = 125)* No. (%)
Fatigue	21 (64)	25 (54)	33 (72)	79 (63)
Abdominal pain	5 (15)	16 (35)	13 (28)	34 (27)
Anorexia	2 (6)	4 (9)	11 (24)	17 (14)
Weight loss	2 (6)	4 (9)	6 (13)	12 (10)
Jaundice	0 (0)	0 (0)	4 (9)	4 (3)
Hepatomegaly	13 (39)	23 (50)	36 (78)	72 (58)
Splenomegaly	3 (9)	2 (4)	18 (39)	23 (18)

*Number of patients in each disease category.
†One patient with cirrhosis presented with hepatocellular carcinoma.

detected in 4 (4%), anti-HBc only in 15 (16%), and both antibodies were present in 23 patients (25%).

Clinical and Laboratory Findings on Presentation

The presenting symptoms and signs of 125 anti-HCV–positive patients are shown in Table 1. Fatigue (63%) was the most common complaint, followed by abdominal pain (27%), anorexia (14%), and weight loss (10%). Jaundice was present in only four patients (3%) with cirrhosis. Hepatomegaly and splenomegaly were detected in 58% and 18%, respectively. The mean presenting laboratory test values included albumin, 42 grams per liter (range, 28 to 54); bilirubin, 15.2 μmol per liter (range, 3.42 to 112.9); aspartate aminotransferase, 100 U per liter (range 18 to 322); and ALT, 156 U per liter (range, 19 to 554).

Liver Disease Classification

After the initial workup, the diagnoses for the 125 anti-HCV–positive patients included 33 (26%) with chronic hepatitis, 46 (37%) with chronic active hepatitis, and 46 (37%) with cirrhosis. When presenting symptoms were assessed by liver disease categories, fatigue was the most common complaint in each disorder (Table 1). In the 46 patients with cirrhosis, 22 (48%) had splenomegaly with thrombocytopenia (mean platelet count, 71.6×10^3 per mm^3; range, 35 to 102), 14 (30%) had ascites, 8 (17%) had esophageal varices, and 4 (9%) had hepatic encephalopathy.

One of the anti-HCV–positive patients with cirrhosis presented with hepatocellular carcinoma. In two other patients with cirrhosis, hepatocellular carcinoma also developed at 18 months and 32 months, respectively, during the follow-up period. The mean AFP level of the three patients with hepatocellular carcinoma was 6,094 μg per liter (range, 1.6 to 17,833). The mean AFP level in all patients without hepatocellular carcinoma was 6.9 μg per liter (range, 1.6 to 120.5). The serum AFP values were consistently normal in patients with chronic hepatitis and with chronic active hepatitis and were above the normal range in 3 (7%) cirrhotic patients without hepatocellular carcinoma (30, 43.7, and 120.5 μg per liter, respectively).

The mean intervals between the time of the initiation of injecting drugs to the time of the diagnosis of either chronic hepatitis, chronic active hepatitis, cirrhosis, or hepatocellular carcinoma are shown in Figure 1. Only the 74 patients who gave a history of a year or less of drug abuse were used for this analysis because it was not possible to predict the time of exposure to HCV in the remaining patients whose time period of self-injecting drugs ranged from 2 to 40 years. Thus, in the above-mentioned 74 patients, the mean number of years from injection drug use to chronic hepatitis was 15.6 years (range 8 to 23 years), to chronic active hepatitis was 17.6 years (range 12 to 24 years), to cirrhosis was 19.4 years (range 11 to 28 years), and it was 26 years (range 18 to 34 years) for the three patients with hepatocellular carcinoma.

Effect of Alcohol Intake on Liver Disease Progression

In an attempt to determine the effect of heavy alcohol consumption on the clinical course of hepatitis C, we interviewed the 74 anti-HCV–positive patients who gave a one-year history or less of injection drug use and divided this cohort into those with and without admitted alcohol abuse. Within this group of 74 patients, 53 (72%) gave a history of heavy alcohol consumption while 21 denied alcohol use. Each patient was asked to estimate the time period of alcohol consumption and the approximate amount of alcohol that was consumed. For those who admitted to heavy alcohol abuse, the time period of use was divided into drinking for 5 years, 6 to 10 years, 11 to 20 years, and for 21 to 40 years. The estimated average number of drinks per day was obtained by history, and the average lifetime consumption of alcohol in kilograms was then calculated. In the 53 patients who admitted to heavy alcohol consumption, the estimated average lifetime consumption of alcohol ranged from 277 kg for those with 5 years of abuse, 471 kg for those with as long as 10 years of abuse, 736 kg for those with as long as 20 years of abuse, and 1,984 kg for the patients with as long as 40 years of alcohol use. In the 21 patients who denied heavy alcoholic use, the estimated average lifetime consumption of alcohol was calculated to be 6.5 kg. When the average time for the development

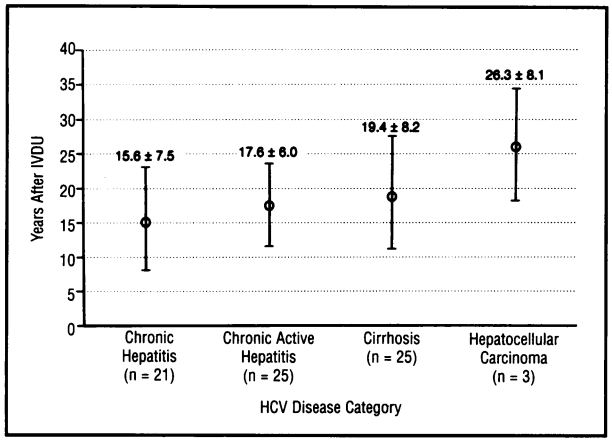

Figure 1.—The graph shows the mean time interval expressed in years plus-minus standard deviation from the time of intravenous drug use (**IVDU**) to presentation with diseases associated with hepatitis C in 74 patients with a 1-year history of intravenous drug use. HCV = hepatitis C virus

of each liver disease category was compared in these 74 patients with and without heavy alcohol abuse, there were no significant time differences noted (14.9 years versus 17.3 years for chronic hepatitis, 18.2 years versus 16.1 years for chronic active hepatitis, and 21.4 years versus 16.9 years for cirrhosis; $P > .20$ in all categories).

Disease Outcome

The overall two-year case-fatality rate in our anti-HCV–positive patients with a history of injection drug use was 2% (3 of 125 patients). One patient died of hepatocellular carcinoma, and two patients died of complications after liver transplantation. Two other patients, one with cirrhosis and one with hepatocellular carcinoma, who also underwent orthotopic liver transplantation are still living at the time of this writing.

Discussion

Hepatitis C infection is a common disease in injection drug users.[1,2] A recent report showed that 71.4% of persons with a history of less than a year of injection drug use were anti-HCV–positive, whereas 91.7% of those who self-injected drugs for more than ten years had antibodies to HCV.[18] These last findings indicate that HCV is commonly transmitted to newer initiates of injection drug use and that a longer duration of use was significantly associated with increasing anti-HCV–positivity. In our report, virtually all (124 of 125) of the anti-HCV–positive patients were HCV RNA–positive. Therefore, it is not surprising that the sharing of needles among injection drug users would result in high rates of HCV transmission.

Hepatitis B infection is also common in injection drug users. In this report, we excluded patients with coexisting hepatitis B infection because the presence of both HBV and HCV may accelerate the rate of liver disease progression. In fact, all three patients with both HBsAg– and anti-HCV–positivity who were excluded from this study had cirrhosis. Past exposure to hepatitis B was evident in this group of injection drug users because hepatitis B antibodies were detected in 45% of our anti-HCV–positive patients. In another report, anti-HBc was detected in 71% and anti-HBs in 51% of injection drug users enrolled in drug treatment programs.[2] Thus, both hepatitis B and C viruses are important pathogens in the injection drug user population.

More than 90% of our patients had detectable antibodies to the HCV recombinant proteins C22 and NS3, 70% had antibodies to NS5 and C100-3, and about 50% had antibodies to the structural proteins E1 and E2. The frequency of antibodies to the individual HCV proteins in our patients with a history of injection drug abuse was similar to our findings in patients with posttransfusion hepatitis C[12] and hence may represent a general pattern of antibody response to these recombinant antigens that is observed after hepatitis C infection regardless of the mode of transmission. The reasons for only a 50% positivity for antibodies to the reputed envelope proteins E1 and E2 are unclear, but they may be due to the fact that these recombinant antigens are linear instead of conformational in configuration.

Our results indicate that fatigue was a common symptom among anti-HCV–positive patients and was present in more than 50% of those with chronic hepatitis, chronic active hepatitis, and cirrhosis. Hepatomegaly was a common finding and was noted more frequently as the liver disease category progressed in severity. These clinical observations are similar to those reported in patients with transfusion-associated hepatitis C.[12] In the latter study, more than 65% of anti-HCV–positive patients presented with fatigue, and hepatomegaly also was frequently detected. Therefore, in our experience at a tertiary care center, patients with hepatitis C commonly presented with symptoms and signs of chronic liver disease. This is in contrast with other reports that indicated that patients with transfusion-associated chronic hepatitis C had few clinical symptoms and signs of liver disease even when observed for 16 to 18 years after HCV infection.[10,11]

In 74 of our patients who gave a history of injection drug use for a year or less, the mean times for the development of chronic hepatitis, chronic active hepatitis, cirrhosis, and hepatocellular carcinoma were 15.6 years, 17.6 years, 19.4 years, and 26.3 years, respectively. These time intervals for the progression of liver disease are similar to those reported for patients with posttransfusion chronic hepatitis C in Japan,[19,20] as well as for transfused patients in the United States.[12] Altogether, these observations indicate that the time of the initial HCV infection may be the major determinant of the type of liver disease that is encountered during an initial presentation to a physician. There are patients, however, who are infected with HCV who do not fit into the above characterizations. Other factors, such as HCV genotypes and viral titers, also may influence the progression of chronic liver disease in these persons.[21-23] One report noted that closely related hepatitis C viral sequences

were detected by polymerase chain reaction in injection drug users, suggesting that they were infected from a common source.[24] Subsequently, specific HCV genotyping of injection drug users showed that types 1a and 3a were more frequently detected in this group of patients in Italy,[25] and genotype 3a was more prevalent in injection drug users in Scotland.[26] A report from Japan suggested that HCV genotypes may not be responsible for the development of serious liver disease because no significant differences in the distribution of any of the genotypes were detected among patients in different stages of chronic liver disease.[27] In one study on the quantitation of serum HCV RNA, injection drug users had a lower median viremia level than patients infected by blood transfusion, and no differences in HCV-RNA values were noted in patients with either chronic hepatitis, chronic active hepatitis, or cirrhosis.[27,28] Other investigators noted, however, that HCV-RNA levels were similar in transfused patients and in injection drug users.[29] The latter report also showed that patients with histologic findings of chronic hepatitis had lower viremia levels than those with chronic active hepatitis or cirrhosis. Further studies are needed to elucidate the role of viral genotypes and of HCV-RNA levels in determining the clinical outcome of injection drug users with chronic hepatitis C infection.

In this report, we attempted to determine the contribution of heavy alcohol abuse to the progression of liver disease in HCV-infected injection drug users. The estimates for the time of alcohol intake and the amount of alcohol consumed relied on memory and on the patients' willingness to even admit to alcohol abuse. For this purpose, we only included patients with a history of one year or less of injection drug use because this is the only group in whom we could predict the alleged time of initial HCV infection. Therefore, 74 patients were interviewed regarding alcohol intake. Of these, 71% admitted to heavy alcohol abuse, and 29% indicated either no alcohol intake or only occasional use. For the patients with heavy alcohol abuse, the estimated total lifetime amount consumed ranged from an average of 277 kg in those who imbibed for 5 years to an average of 1,984 kg for those who imbibed for as long as 40 years. These values were compared with a lifetime consumption of an average of only 6.5 kg in the patients who said they drank little or no alcohol. Using the above measures, we found no differences in the time of progression to chronic hepatitis, chronic active hepatitis, or cirrhosis when patients with a history of prolonged alcohol intake were compared with those without a drinking history. Based on these findings, the excessive use of alcohol in this subgroup of patients did not appear to hasten the progression of liver disease when compared with those with little or no alcohol consumption. Other reports showed a high prevalence of anti-HCV–positivity in patients with alcoholic liver disease, and it was suggested that HCV infection may have accelerated the progression to cirrhosis in these persons.[16,30-32] In our patients, however, chronic hepatitis C infection from injection drug use probably was the primary cause of their liver disease, and their alcohol intake may not have been as heavy or as prolonged as those patients in previous reports who were considered to have established alcoholic liver disease and then incidentally found to be anti-HCV–positive. Further studies are needed to clarify the association between chronic hepatitis C infection and alcohol intake.

In this report of hepatitis C infection associated with injection drug use, the mortality rate was 2%, and hepatocellular carcinoma developed in three patients. The average age of our patients at presentation to a tertiary care center, however, was relatively young (43.5 years), and the average time period from initial HCV infection was about 20.4 years. The relatively low morbidity and mortality in our patients is similar to those reported for patients with posttransfusion hepatitis C who had been observed for periods of 16 and 18 years.[10,11] Therefore, it is conceivable that more of our patients will progress to serious liver disease or hepatocellular carcinoma as they advance in age and as the time of the HCV infection becomes more prolonged. Such was the observation in our patients with transfusion-associated chronic hepatitis C.[12] Whether these outcomes will be the case or whether the course of hepatitis C infection is less severe and has a slower progression in patients who acquire their HCV from injection drug use rather than by transfusion[8,9] will only be clarified with long-term follow-up of these persons.

In summary, our findings indicate that hepatitis C associated with injection drug use is a progressive disease, and serious liver complications may develop with time. Both proper education concerning the transmission of HCV in these patients and practical measures to prevent infection must be continued by public health officials. Finally, antiviral therapy should be attempted to eliminate the virus and to prevent progression of this chronic viral illness.

REFERENCES

1. Kelen GD, Green GB, Purcell RH, et al: Hepatitis B and hepatitis C in emergency department patients. N Engl J Med 1992; 326:1399-1404

2. Zeldis JB, Jain S, Kuramoto IK, et al: Seroepidemiology of viral infections among intravenous drug users in northern California. West J Med 1992; 156: 30-35

3. Esteban JI, Esteban R, Viladomiu L, et al: Hepatitis C virus antibodies among risk groups in Spain. Lancet 1989; 2:294-297

4. Mortimer PP, Cohen BJ, Litton PA, et al: Hepatitis C virus antibody (Letter). Lancet 1989; 2:798

5. Bell J, Batey RG, Farrell GC, Crewe EB, Cunningham AL, Byth K: Hepatitis C virus in intravenous drug users. Med J Aust 1990; 153:274-276

6. van den Hoek JAR, van Haastrecht HJA, Goudsmit J, de Wolf F, Coutinho RA: Prevalence, incidence, and risk factors of hepatitis C virus infection among drug users in Amsterdam. J Infect Dis 1990; 162:823-826

7. Alter MJ: The detection, transmission, and outcome of hepatitis C virus infection. Infect Agents Dis 1993; 2:155-166

8. Mattsson L, Weiland O, Glaumann H: Chronic non-A, non-B hepatitis developed after transfusions, illicit self-injections or sporadically—Outcome during long-term follow-up: a comparison. Liver 1989; 9:120-127

9. Gordon SC, Elloway RS, Long JC, Dmuchowski CF: The pathology of hepatitis C as a function of mode of transmission: Blood transfusion vs. intravenous drug use. Hepatology 1993; 18:1338-1343

10. Koretz RL, Abbey H, Coleman E, Gitnick G: Non-A non-B post-transfusion hepatitis—Looking back in the second decade. Ann Intern Med 1993; 119:110-115

11. Seeff LB, Buskell-Bales Z, Wright EC, et al: Long-term mortality after transfusion-associated non-A non-B hepatitis—The National Heart, Lung, and Blood Institute Study Group. N Engl J Med 1992; 327:1906-1911

12. Tong MJ, El-Farra NS, Reikes AR, Co RL: Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995; 332:1463-1466

13. Boyer JL: Chronic hepatitis—A perspective on classification and determinants of prognosis. Gastroenterology 1976; 70:1161-1171

14. Ludwig J: The nomenclature of chronic active hepatitis: An obituary. Gastroenterology 1993; 105:274-278

15. Tong MJ, Lee SY, Hwang SJ, et al: Evidence for hepatitis C viral infection in patients with primary hepatocellular carcinoma. West J Med 1994; 160:133-138

16. Caldwell SH, Li X, Rourk RM, et al: Hepatitis C infection by polymerase chain reaction in alcoholics: False-positive ELISA results and the influence of infection on a clinical prognostic score. Am J Gastroenterol 1993; 88:1016-1021

17. Chien DY, Choo QL, Tabrizi A, et al: Use of recombinant HCV antigen in the serodiagnosis of hepatitis C virus infection: Significant improvement in HCV antibody detection as compared with the first generation HCV C100-3 ELISA and the synthetic peptide EIA tests. J Gastroenterol Hepatol 1993; 8:S33-S39

18. Donahue JG, Nelson KE, Muñoz A, et al: Antibody to hepatitis C virus among cardiac surgery patients, homosexual men, and intravenous drug users in Baltimore, Maryland. Am J Epidemiol 1991; 134:1206-1211

19. Kiyosawa K, Sodeyama T, Tanaka E, et al: Interrelationship of blood transfusion, non-A non-B hepatitis and hepatocellular carcinoma: Analysis by detection of antibody to hepatitis C virus. Hepatology 1990; 12(pt 1):671-675

20. Takahashi M, Yamada G, Miyamoto R, Doi T, Endo H, Tsuji T: Natural course of chronic hepatitis C. Am J Gastroenterol 1993; 88:240-243

21. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al: Hepatitis C virus genotypes: An investigation of type-specific differences in geographic origin and disease. Hepatology 1994; 19:13-18

22. Mita E, Hayashi N, Kanazawa Y, et al: Hepatitis C virus genotype and RNA titer in the progression of type C chronic liver disease. J Hepatol 1994; 21:468-473

23. Nousbaum JB, Pol S, Nalpas B, et al: Hepatitis C virus type 1b (II) infection in France and Italy. Ann Intern Med 1995; 122:161-168

24. Simmonds P, Zhang LQ, Watson HG, et al: Hepatitis C quantification and sequencing in blood products, haemophiliacs, and drug users. Lancet 1990; 336:1469-1472

25. Silini E, Bono F, Cividini A, et al: Molecular epidemiology of hepatitis C virus infection among intravenous drug users. J Hepatol 1995; 22:691-695

26. Chan SW, McOmish F, Holmes EC, et al: Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants. J Gen Virol 1992; 73(pt 5):1131-1141

27. Yamada M, Kakumu S, Yoshioka K, et al: Hepatitis C virus genotypes are not responsible for development of serious liver disease. Dig Dis Sci 1994; 39: 234-239

28. Lau JYN, Davis GL, Kniffen J, et al: Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet 1993; 341:1501-1504 [erratum published in Lancet 1993; 342:504]

29. Gordon SC, Kodali VP, Silverman AL, et al: Levels of hepatitis C virus RNA and liver histology in chronic type C hepatitis. Am J Gastroenterol 1994; 89:1458-1461

30. Poynard T, Aubert A, Lazizi Y, et al: Is hepatitis C virus infection a cause of cirrhosis among drinkers? *In* Hollinger FB, Lemon SM, Margolis HS (Eds): Viral Hepatitis and Liver Disease—International Symposium on Viral Hepatitis and Liver Disease: Contemporary Issues and Future Prospects. Baltimore, Md, Williams & Wilkins, 1990, pp 681-683

31. Laurent-Puig P, Dussaix E, Lecoz Y, Martes P, Buffet C: Prevalence of anti-hepatitis C virus antibodies among patients with alcoholic liver disease, supplemented by 4-RIBA (Letter). Dig Dis Sci 1992; 37:156-157

32. Zarski JP, Thelu MA, Moulin C, Rachail M, Seigneurin JM: Interest of the detection of hepatitis C virus RNA in patients with alcoholic liver disease. J Hepatol 1993; 17:10-14